

Distribution, Metabolism, and Excretion of Di-2-ethylhexyl Phthalate in the Rat*

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It has become increasingly evident in recent years that the dialkyl phthalate esters are widely distributed in our environment (1-4). As an explanation of this wide distribution there are many possible pathways by which these chemicals can be introduced into the human body. Jaeger and Rubin (5) have shown that di-2-ethylhexyl phthalate (DEHP) can be found in the tissues of patients who have received transfusions of blood stored in plastic bags. Moreover, the possibility of introducing significant quantities of phthalate esters to the system through ingestion of foodstuffs contaminated with these materials cannot be ignored. In order more fully to understand the toxicological implications of phthalate ester contamination, it is important to determine the fate of these materials once they are within the body. The study described here was undertaken in order to ascertain the distribution, metabolism, and elimination of DEHP administered to rats.

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Materials and Methods

Materials

Di-2-ethylhexyl phthalate (DEHP) labeled with carbon-14 at the carbonyl carbon was obtained from American Radiochemicals Corporation, Sanford, Florida. Unlabeled DEHP was provided by the Monsanto Company, St. Louis, Missouri. The rats used in this study were adult (150-250 g) male CFN rats from Carworth Farms, Inc., New York, New York. Radioactivity assays were performed by using a Packard Tri-Carb Model 3200 scintillation spectrometer. Thin-layer chromatographs were run on Brinkman silica gel G-25 UV₂₅₄ plates.

Procedure

Labeled DEHP was prepared for intravenous injection by dissolving labeled and unlabeled DEHP in hexane. An appropriate amount of this hexane solution was transferred to a glass rosette vessel, and the hexane solvent was removed by evaporation. The volume was made up to 10 ml with a solution of 4% bovine serum albumin in 0.9% aqueous sodium chloride. The mixture was then probe sonicated for 10 min with a Branson sonifier using the microprobe tip. Stability of the resulting solution or emulsion was monitored by assaying aliquots for homogeneous dispersion of the radioactivity using the scintillation spectrometer. Two different injection mixtures were prepared by this method. The preparation used for

the low dose contained 0.02 mg DEHP/ml and 5 μ Ci/ml. This preparation, following sonication, was clear and thus presumably represents a true solution. It was injected via the dorsal penis vein on the basis of 5 ml/kg of body weight, resulting in a dose of 0.1 mg DEHP/kg. The preparation used for the high dose contained 40 mg DEHP/ml and 5 μ Ci/ml. Sonication of this preparation resulted in a milky emulsion. Administration of 5 ml of this emulsion/kg of body weight resulted in a dosage of 200 mg DEHP/kg of body weight.

For oral administration, DEHP was prepared in corn oil at a concentration of 40 mg/ml. Intubation of 5 ml/kg of body weight resulted in an oral dose of 200 mg/kg.

After administration of the dose, the animals were housed in metabolism cages so that the urine and feces could be collected. The rats were allowed free access to food and water. Blood samples were taken from the tail artery. After 1 or 24 hr, the animals were sacrificed by decapitation and were dissected. Any urine remaining in the urinary bladder was withdrawn with a syringe and combined with the collected urine. The blood, urine, feces, and tissues were then analyzed according to the scheme outlined in Figure 1. Subsequent treatment of the aqueous phase with chloroform-methanol extracted no additional radioactivity, indicating that the first extraction step was

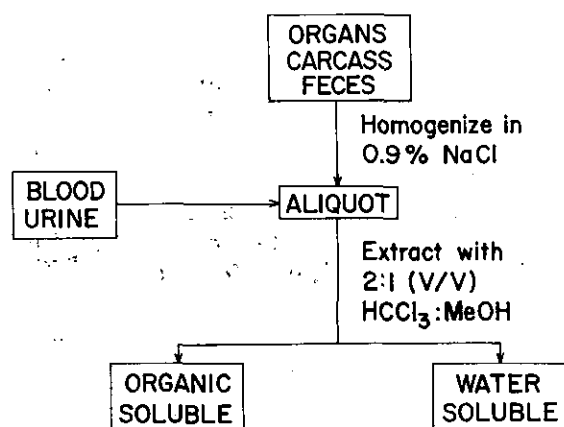


FIGURE 1. Analytical scheme for the determination of radioactivity of tissue samples from rats.

complete and that any residual radioactivity is associated with a water-soluble material.

Thin-layer chromatograms were developed by using a 65:25:4 (v/v/v) mixture of chloroform, methanol, and water. The developed plates were visualized under ultraviolet light. Distribution of radioactivity was determined by scraping and collecting 0.5-cm wide strips of the silica gel, progressing vertically from the origin to the solvent front. The scrapings were individually analyzed for radioactivity in the scintillation spectrometer.

Results and Discussion

The very rapid logarithmic disappearance of organic extractable radioactivity from the bloodstream, which is assumed to correspond to the disappearance of unchanged DEHP, is shown in Figure 2. The disappearance appears to be biphasic. The initial rapid phase corresponds to a half-time of 9.0 min for unchanged DEHP and continues for about 30 min. Thus, after 30 min, less than 15% of the material initially present in the blood is still present in the bloodstream. The second, less rapid phase corresponds to a half-time of 22 min. Such

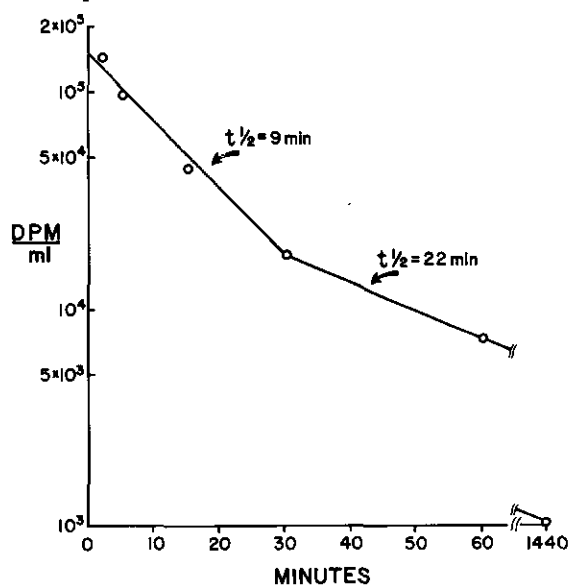


FIGURE 2. Kinetics of DEHP disappearance from the blood of rats following a 200 mg/kg intravenous dose.

biphasic behavior is not unusual and results from two processes having different rate constants.

Figure 3 shows the time course of disappearance of organic extractable radioactivity in the blood of rats receiving the low dose (0.1 mg/kg) of DEHP intravenously. It will be noted that the initial rate of disappearance is even faster at the low dose than at the high dose ($t_{1/2} = 4.5$ min). The rapid initial rates of disappearance at both doses and the more rapid rate at the low dose strongly suggest a selective uptake of DEHP by some tissue compartment with saturation of that uptake process occurring at the larger dose. The slower, second phase of disappearance of the low dose from the blood is represented by a dashed line in Figure 3, since there are insufficient data to determine the actual slope. The slope of the dashed line is the same as that for the slow phase of disappearance at the high dose. Whether this assumption is valid or not, it is clear that the 60 min data lie on a slope distinctly different from the initial slope.

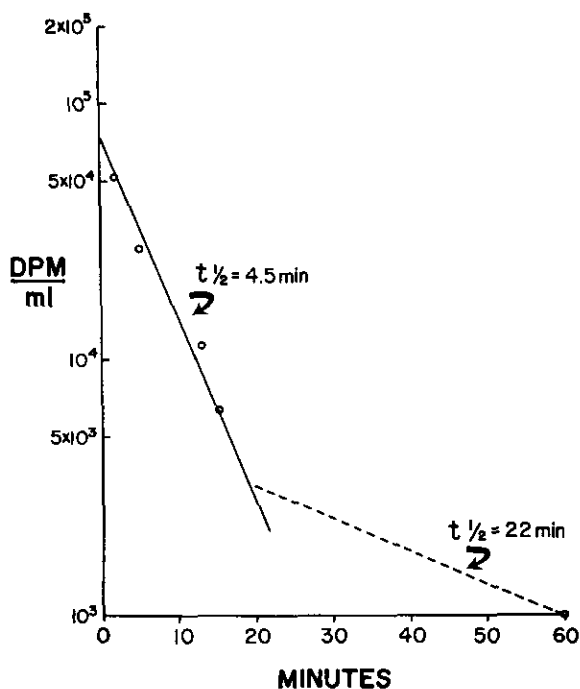


FIGURE 3. Kinetics of DEHP disappearance from the blood of rats following a 0.1 mg/kg intravenous dose.

Figure 4 shows the buildup of a small but significant amount of water-soluble radioactive label in the bloodstream after administration of either dose. This buildup is consistent with the hypothesis that the water-soluble material is a metabolite or metabolites of DEHP.

Additional support for the concept of accumulation of DEHP at specific locations against a concentration gradient is given by calculating apparent volumes of distribution V_d . Fifteen minutes after injection of the low dose, before metabolism and excretion have become significant, the V_d is 341 ml, well in excess of the volume of total body water.

Figure 5 shows the distribution of radioactive label among tissues and excretory products 1 and 24 hr after the intravenous injection of 200 mg DEHP/kg. At 1 hr, 71.4% of the injected dose was recovered in the organic extractable fraction and another 8.0% in a water-soluble form. With regard to tissue localization, approximately 50% of the injected dose was found in the liver after 1 hr. This observation is consistent with a site of selective uptake of DEHP suggested by the kinetics of its disappearance from blood. The only other organs, in addition to the carcass, which contain significant levels of organic extractable label after 1 hr are those organs of known reticuloendothelial function (i.e., the spleen and lungs). The presence of significant levels of organic extractable material in the large intestine (including the

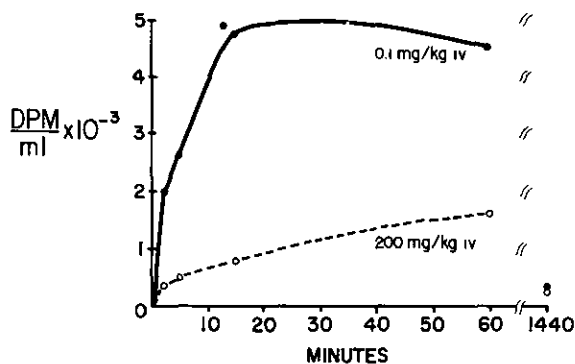


FIGURE 4. Appearance of water-soluble derivatives of DEHP in the blood: (—) 0.1 mg/kg intravenous dose; (---) 200 mg/kg intravenous dose.

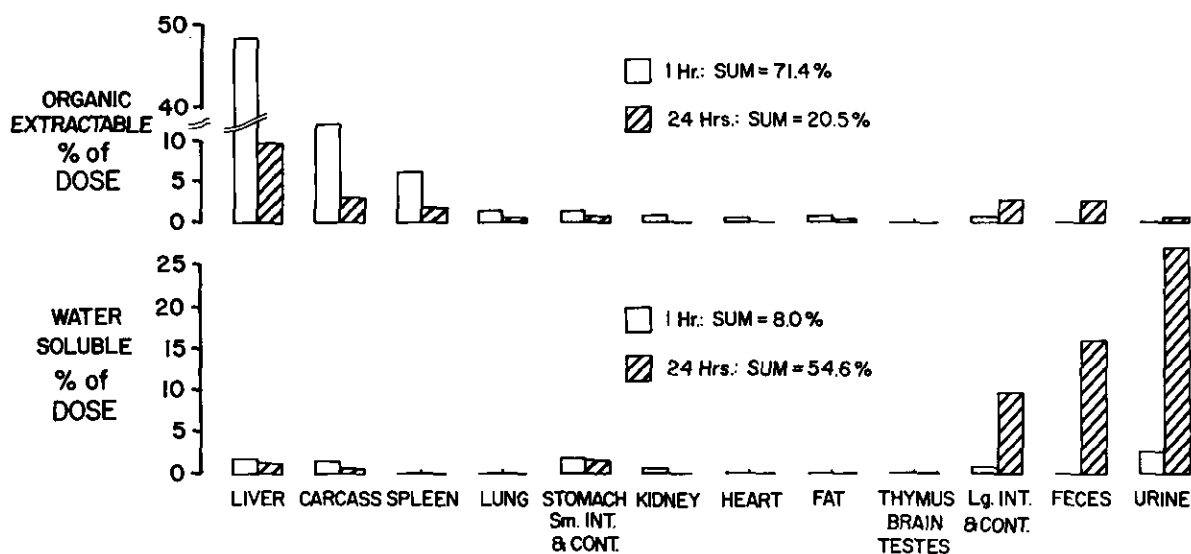


FIGURE 5. Tissue distribution of DEHP as percentage of the injected dose: (□) 1 hr and (▨) 24 hr after administration of a 200 mg/kg intravenous dose. Flat bars represent trace amounts (less than 0.1% of the injected dose).

contents) indicates that unchanged DEHP might enter the gastrointestinal tract by some secretory process, presumably biliary.

The 8.0% of the total injected dose that is found in the water-soluble form after 1 hr is distributed primarily in the liver, the intestinal contents, and the urine. These data suggest that the liver is the site of the metabolism of the original DEHP to a water-soluble form and that this metabolite is rapidly excreted into the gastrointestinal tract and the urine. This suggestion is further strengthened by the distribution of the radioactivity after 24 hr. At this time, 54.6% of the total injected dose can be recovered in the water-soluble fraction, primarily in the intestinal contents, excreted feces, and the urine, while only 20.5% of the dose can be recovered in the organic extractable form. It is interesting to note that over the 24-hr period, the organic extractable radioactivity fell in all organs and increased in the large intestine, feces, and urine, thus indicating biliary and renal excretion as additional routes of elimination of DEHP.

Earlier experiments in our laboratory using emulsions prepared with a less powerful sonicator indicated that one third of the injected dose (200 mg/kg) was retained as

unchanged DEHP in the lungs after twenty-four hours. These animals continued to exhibit significant quantities of DEHP in their lungs up to 24 days after injection. Since the present studies show only 0.3% of the injected dose in the lungs after 24 hr, it is clear that the particle size of the DEHP in the injection medium is critical for its distribution within the body.

The distribution of label 1 hr after an intravenous injection of a 0.1 mg/kg dose and of a 200 mg/kg dose is compared in Figure 6. Qualitatively, the distribution of label among the tissues is similar at the low

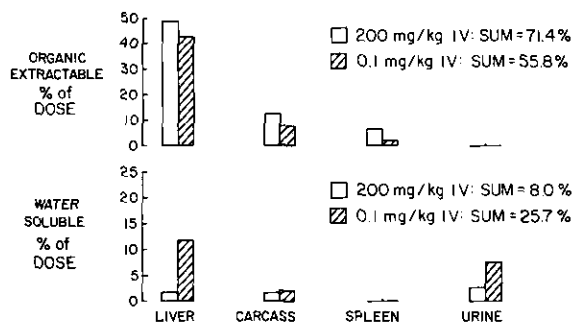


FIGURE 6. Comparison of the tissue distribution of two different intravenous doses of DEHP 1 hr after injection: (□) 200 mg/kg dose; (▨) 0.1 mg/kg dose. Flat bars represent trace amounts (less than 0.1% of the injected dose).

dose to that for the higher dose. However, the percentage of the injected dose recovered as unchanged DEHP is slightly lower at each site for the lower dose and totals only 55.8% as compared to 71.4% unchanged at the higher dose. The most striking difference is that, whereas after 1 hr only 8% of the higher dose has been converted to water-soluble metabolite, 25% of the lower dose has undergone this conversion. These results apparently reflect saturation of the metabolic mechanism by the higher dose, with the result that a correspondingly smaller percentage of the higher dose is metabolized.

In Figure 7 is shown the distribution data from rats given the low dose of DEHP. At this dose level essentially all the injected material has been converted to water-soluble metabolites and excreted in the urine and feces after 24 hr. Only a small percentage (1-2%) of the original dose remains in the body under these conditions.

The results of oral administration of DEHP in corn oil by gastric intubation are summarized in Table 1. It can be seen in the lower portion of Table 1 that approximately 80% of the intubated dose can be recovered after 24 hr. Of this, 61.7% was found in the water-soluble fraction, either excreted in the

urine and feces or in the large intestine, presumably in the fecal material. The remaining 12.8% was found as organic-soluble DEHP, and once again, in the excreta. It is important to note that negligible radioactivity was found in all organs assayed.

In the light of this extensive metabolism of an oral dose of DEHP, it is interesting to note the extremely small amounts of radioactivity that appear in the blood following the oral intubation. The data are presented here as disintegrations per minute per milliliter (dpm/ml) of the whole blood and their magnitude should be compared to the 3.337×10^6 dpm of DEHP administered. It is also interesting that, in spite of the low levels of radioactivity recovered in the blood, the level of water-soluble radioactivity at each time point is greater than the organic-extractable. These results indicate that the metabolism of DEHP is extensive and that the rate of metabolism is more rapid than the rate at which it is absorbed from the gastrointestinal tract.

In order to understand the nature of the metabolism of DEHP more fully, attempts have been made to isolate and characterize the water-soluble labeled material in the feces and urine from the experimental animals. It has been found that if the aque-

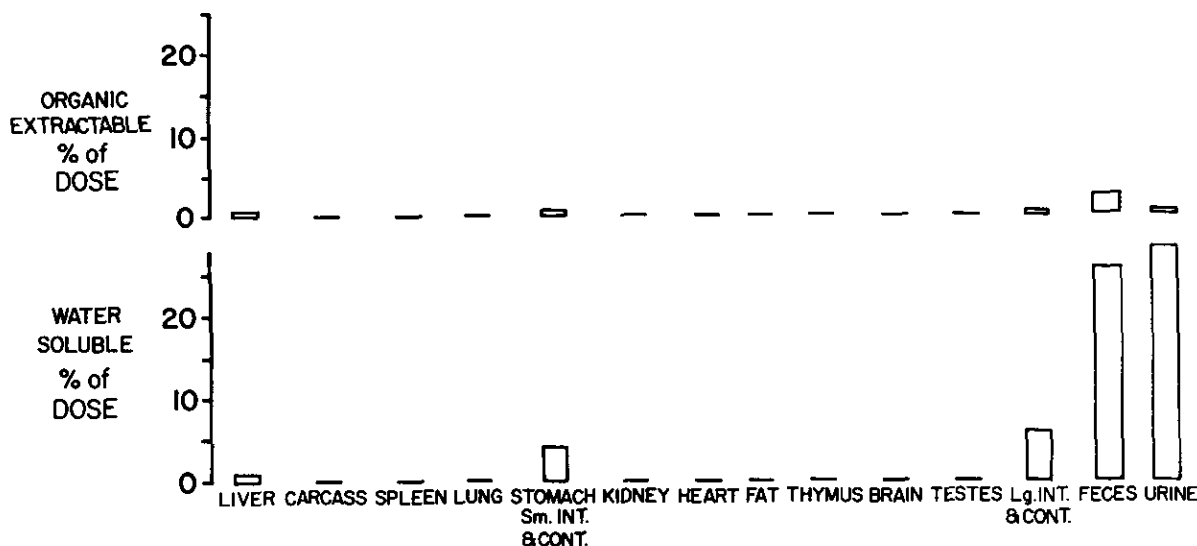


FIGURE 7. Tissue distribution of DEHP 24 hr after administration of a 0.1 mg/kg intravenous dose. Flat bars represent trace amounts (less than 0.1% of the injected dose).

Table 1. Absorption and distribution of an oral dose of 200 mg/kg of DEHP in corn oil administered by gastric intubation.

Time, hr	Site	Blood level, dpm/ml ^a		Distribution, % of administered dose	
		Organic	Aqueous	Organic	Aqueous
1	Blood	20	577		
3	Blood	76	752		
5	Blood	54	811		
24	Blood	10	218		
24	Urine			0.3	33.9
24	Feces			9.9	13.0
24	Large intestine and contents			2.5	14.6
24	Small intestine and contents			0.1	0.2
24	All other organs			Negligible	Negligible
24	Total			12.8	61.7

^a3.337 x 10⁶ dpm administered in corn oil.

ous layer resulting from the extraction of urine or feces is acidified to pH < 1 with concentrated hydrochloric acid, more than 95% of the labeled material can then be extracted from the aqueous phase by using chloroform. This behavior is consistent with the presence of an ionizable functional group in the water-soluble material. When the chloroform extract was concentrated and analyzed by thin-layer chromatography there was a complex array of spots distributed from the origin to the solvent front. However, an analysis of the distribution of radioactivity on the plate gave a pattern like that shown in Figure 8. It is clear from this figure that there are at least four different major metabolites. The spots at the top of the figure represent the relative mobilities of phthalic acid, 2-ethylhexyl acid phthalate (the monoester of DEHP), and DEHP. The lack of correspondence of any of these markers with the radioactive spots indicates that metabolism is not a simple stepwise hydrolysis of the diester. Comparable thin-layer chromatograms of nonfractionated urine were not substantially different from those of the chloroform extract. The same is true of urine collected directly from the urinary bladder of rats 1 hr after administration of the DEHP, eliminating the possibility of microbial metabolism as being responsible for the appearance of the water

soluble metabolites. Attempts to isolate significant quantities of each major metabolite by column chromatography on a silicic acid gel were unsuccessful due to insufficient separation, indicating that chemically these compounds may be very similar. Small quantities of three of the major metabolites have been isolated by preparative thin layer chromatography. Ultraviolet spectra of these materials in methanol gave an absorption maximum between 277 and 280 nm for each compound, indicating a similar conjugated framework in each. Mass spectra of these compounds obtained at an ionizing voltage of 80 eV gave a fragmentation pattern in which almost all the peaks were below molecular weight 100. The only generalization which can be made is that the pattern is similar to that for compounds containing a benzenoid ring structure.

Currently, attempts are being made to separate the major metabolites by gas chromatography so that they can be characterized by combined GC-mass spectral analysis.

Summary

The experiments described in this paper show that in rats intravenous DEHP dis-

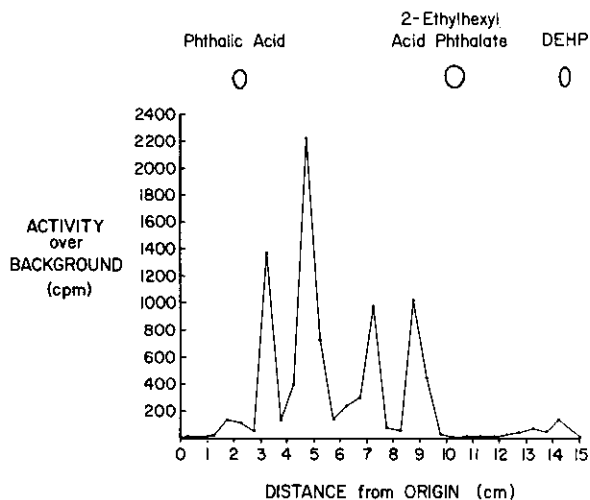


FIGURE 8. Radioactivity profile of a thin-layer chromatograph of the water-soluble metabolites extracted with chloroform from the acidified urine of rats given an intravenous dose of ¹⁴C-labeled DEHP.

appears rapidly from the bloodstream and is localized primarily in the liver. This occurs whether the DEHP is administered as a solution or as an emulsion. In addition, depending upon the degree of emulsification, varying amounts of DEHP can be found in the lungs, ranging from less than 2% after 24 hr for finely emulsified preparations to greater than 30%, with long-term retention, for poorly emulsified preparations. Thus, the clearance of DEHP by the pulmonary circulation is highly dependent upon the dimensions of the oil droplets presented to it. On the other hand, clearance by the liver appears to occur readily, independent of the degree of emulsification. However, the hepatic uptake process appears to be saturated at a dose of 200 mg/kg as suggested by the slower rate of disappearance of this higher dose from the blood.

In addition, DEHP appears to be extensively metabolized by the rat to water-soluble metabolites which are excreted primarily in the urine and feces. Thus an intravenous dose of 0.1 mg DEHP/kg of body weight can be almost totally accounted for as excreted water-soluble metabolites within 24 hr of administration.

Following oral administration of 200 mg/kg, the metabolism of DEHP and excretion of the metabolites are so rapid relative to the rate of absorption of DEHP from the gastrointestinal tract that no significant quantities of DEHP can be found in the

blood or tissues. Finally, preliminary results have shown that metabolism of DEHP by the rat does not consist of simple stepwise de-esterification of the dialkyl ester. Rather, at least four chemically similar water-soluble metabolites are formed.

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